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Regulation of inositol transport by glucose and protein kinase C in mesangial cells

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Regulation of inositol transport by glucose and protein kinase C in mesangial cells. Since inositol (Ins) depletion appears to be an important mechanism of cell injury in diabetic glomerulopathy, we studied Ins transport in cultured rat mesangial cells during hyperglycemia. High glucose stimulated [^{3}H]-Ins uptake by 50 to 90% within 24 hours in a dose dependent manner. This effect was characterized by an increase in the V_{max} of a Na^+-dependent Ins transporter (10.3 ± 0.2 vs. 16.4 ± 0.4 pmol/mg/min, $P < 0.005$). Since high glucose also induced activation of protein kinase C (PKC) in permeabilized mesangial cells, we examined the potential role of this enzyme in the stimulation of Ins transport by glucose. Both PKC inhibition with H7 and staurosporine, and down regulation of PKC by prolonged PMA (1.6 μM) treatment inhibited the stimulatory effect of glucose on Ins transport. In conclusion, high glucose stimulates Na^+-dependent Ins transport in mesangial cells by a mechanism mediated by PKC. This process may represent an important adaptive response of mesangial cells to hyperglycemia.

The importance of *myo*-inositol (Ins) as a growth factor for mammalian cells and of inositol lipids as critical mediators of transmembrane signal transduction processes is now widely recognized [1–3]. Ins deficiency can lead to alterations in cell growth and abnormal phosphoinositide metabolism [1, 4, 5]. Intracellular Ins depletion has been observed in a variety of tissues during diabetes mellitus and appears to be an important factor in the pathogenesis of diabetic injury. Studies in streptozotocin-induced diabetic rats have shown that the content of Ins in sciatic nerve, autonomic superior cervical ganglion and renal glomerulus is reduced as a result of hyperglycemia [6–9]. Moreover, pharmacologic dietary Ins supplementation administered to otherwise untreated streptozotocin-induced diabetic rats has been shown to lead to restoration of both sciatic nerve Ins content and pathologically slowed nerve conduction velocity to normal [10]. Emphasizing the importance of Ins depletion in diabetes mellitus, several recent studies suggest that the developmental abnormalities and birth defects observed in offspring of diabetic mothers may be due to impairment of Ins uptake by glucose resulting in Ins depletion in the conceptus [11–13].

Inositol depletion due to inhibition of Ins uptake by high concentrations of glucose has also been observed in cultured glomerular mesangial cells [4]. Ins deficiency in mesangial cells leads to abnormal phosphoinositide metabolism and cellular dysfunction, which may underlie the abnormal glomerular contractility and excessive mesangial matrix production observed in early diabetic nephropathy [5, 14, 15]. A number of recent studies have extensively characterized the competitive inhibition of Ins uptake by glucose in a variety of cells [4, 16–23]. Little is known, however, about the intracellular mechanisms that regulate Ins transport under physiologic and pathologic conditions and the adaptive and compensatory responses that occur during Ins deficiency. In this study, we investigate the effects of high concentrations of glucose on the activity of the Ins transporter and the mechanisms that mediate these effects. We report that in rat glomerular mesangial cells in culture, hyperglycemia causes upregulation of Ins transport by a mechanism involving glucose-induced activation of protein kinase C.

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Methods

Sprague-Dawley rats were obtained from the University of Florida animal breeding facilities. Insulin-transferrin-selenium (ITS), D-glucose, D-mannitol and D-*myo*-inositol were obtained from Sigma Chemical (St. Louis, Missouri, USA). RPMI 1640 and minimum essential medium, fetal bovine serum, penicillin and streptomycin were purchased from Fisher Scientific (Orlando, Florida, USA). [^{3}H]-myo-inositol was purchased from New England Nuclear (Boston, Massachusetts, USA). H7 was obtained from Boehringer, staurosporine from Calbiochem and phorbol myristate acetate (PMA) from Avanti Polar Lipids. The peptide QKRPSQRSKYL (MBP_{4-14}) was synthesized by the Protein Chemistry Core Facility, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida.

Preparation of mesangial cell cultures

All experiments were performed in cultured rat mesangial cells between passages 2 and 12. Mesangial cells were harvested from male Sprague-Dawley rats weighing between 150 g and 200 g following previously published techniques [24, 25]. Mesangial cell cultures were prepared after glomerular isolation by mechanical sieving using standard aseptic techniques. The isolated glomeruli were digested for 30 minutes with type IV collagenase (750 U/ml) at 37°C and the glomerular "cores," which contain mostly mesangial and endothelial cells, were

plated in tissue culture flasks and incubated at 37°C in a 5% CO_2/95% air incubator. The culture medium consisted of RPMI 1640 containing 16% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml selenium. After three to four weeks, the mesangial cells overgrow the other cells, become confluent and constitute more than 90% of the cells in the culture. After two passages, no endothelial or epithelial cells were seen and the cultures consisted exclusively of mesangial cells. Mesangial cells were identified using the following characteristics [24–26]: (1) spindle or stellate shape under phase contrast microscopy; (2) presence of microfilament bundles under transmission electron microscopy; (3) presence of immunofluorescence staining for anti-smooth muscle specific myosin antibodies; (4) resistance to the effects of the glomerular epithelial cytotoxin, aminonucleoside of puromycin (100 μg/ml); (5) absence of immunofluorescence staining for anti-factor VIII antibodies which excludes the presence of endothelial cells; and (6) contractile responses to angiotensin II and norepinephrine.

Experimental conditions

In preparation for an experiment, mesangial cells were grown to semiconfluence in 35 mm dishes for [^{3}H]-Ins uptake, or in 96-well plates for protein kinase C determinations, and growth was arrested by 72 to 96 hours of incubation in minimum essential medium (MEM) containing 0.5% fetal bovine serum. At the same time, insulin was withdrawn and *myo*-inositol was adjusted to maintain a concentration of 30 μM (total concentration taking into account *myo*-inositol present in serum) [27]. At various times before experiments, mesangial cells matched for cell line, number of passages and age were divided into two groups: one control group was incubated in MEM as above with 5 mM glucose and one experimental group was incubated in the same medium but with 30 mM glucose (high glucose). The tissue culture medium was replaced daily. In some experiments, a second experimental group was used as control for osmolarity. These cells were incubated in the above medium containing 5 mM glucose and 25 mM mannitol (high mannitol). The osmolarities were 282 to 300 mOsm/kg H_2O in the control, 300 to 320 mOsm/kg H_2O in the high glucose and 300 to 316 mOsm/kg H_2O in the high mannitol media. Cell viability, as assessed by morphologic characteristics of the culture (absence of cell detachment, vacuolization or abnormal granularity) and trypan blue exclusion, was judged to be greater than 95% under all conditions studied.

Determination of myo-*inositol uptake*

After predetermined periods of exposure to normal or high glucose, mesangial cells were washed with HEPES-salt buffer (HSB) (in mM: HEPES 20, NaCl 140, KCl 5, $CaCl_2$ 1, $MgSO_4$ 2.5, *myo*-inositol 0.05 and glucose 5, pH 7.40), incubated with 2 μCi/ml [^{3}H]-Ins (10 to 20 Ci/mmol) at 37°C, and Ins uptake measured at the times indicated. In agreement with what has been previously described in the literature, Ins uptake was found to be linear for over 30 to 60 minutes [4]. Ins uptake was terminated by washing the cells with ice-cold PBS and adding 0.5 N NaOH. Aliquots were used for scintillation counting and protein determination by the Lowry assay [28]. Na^+-dependent, specific Ins uptake was calculated as the difference between uptake in HSB and uptake in Na^+-free HSB containing 140 mM choline Cl, and constituted 92 to 98% of total uptake. For kinetic analysis of Ins uptake, Na^+-dependent [^{3}H]-Ins uptake was measured in the presence of 0.2 to 96 μM Ins, and the V_{max} and K_m were determined by reciprocal (Lineweaver-Burke) plots.

Measurement of protein kinase C activity

Protein kinase C activity was measured in permeabilized mesangial cells using a modification of a previously described assay based on the phosphorylation of a synthetic peptide substrate corresponding to the phosphorylation site for protein kinase C in myelin basic protein (QKRPSQRSKYL, MBP_{4-14}) [29, 30]. Mesangial cells exposed to 5 or 30 mM glucose for 24 or 48 hours were washed with HBS and incubated in 40 μl of permeabilization/kinase assay buffer for 15 minutes at 30°C. This buffer contains NaCl 137 mM, KCl 5.4 mM, Na phosphate 0.3 mM, K phosphate 0.4 mM, glucose 1 mg/ml, HEPES 20 mM (pH 7.2 at 30°), digitonin 50 μg/ml, $MgCl_2$ 10 mM, β-glycerophosphate 25 mM, [γ-^{32}P] ATP 100 μM (approx. 5000 cpm/pmol), MBP_{4-14} 100 μM, EGTA 5 mM and $CaCl_2$ 2.5 mM. After 15 minutes the reaction was terminated with 10 μl of 25% (wt/vol) trichloroacetic acid and 45 μl of the acidified reaction mixture were spotted on 2 × 2-cm phosphocellulose filter squares (Whatman P-81). These were then washed batchwise in three changes (500 ml each) of 75 mM phosphoric acid and one change of 75 mM sodium phosphate (pH 7.5), and counted for radioactivity. Results are expressed as fmol of MBP_{4-14} peptide phosphorylated per mg protein per 15 minutes.

Protein kinase C inhibition, activation and depletion

For this group of experiments, mesangial cells were incubated in 5 or 30 mM glucose in the presence or absence of the protein kinase C inhibitors H7 (10 μM) or staurosporine (10 nM). Direct PKC activation with low concentrations of PMA (10 nM) and downregulation by prolonged (16 hr) incubation with 1.6 μM PMA were also used to confirm the role of PKC in mediating the observed changes in Ins transport.

Statistical analysis

Results are presented as mean ± standard error of the indicated number of experiments performed in triplicate in four different cell lines. Statistical analysis was performed using the Student's *t*-test for paired and unpaired data, or analysis of variance (ANOVA) and subsequent Scheffe's F-test (StatView™ II, Abacus Concepts, Inc. Berkeley, California, USA) as appropriate.

Results

Effects of glucose on myo-*inositol uptake*

To determine the effects of high glucose on Ins uptake, mesangial cells were incubated under normal (5 mM) or high (30 mM) concentrations of glucose over periods of time ranging from 1 hour to 10 days and Ins uptake was measured as described above. Incubation in high glucose resulted in a significant increase in the initial rate of Ins uptake which was initially observed after four to six hours (12 to 19% increase) and reached maximum within 24 to 48 hours (Fig. 1). This maximum increase ranged in magnitude from 50 to 90%, was sustained for as long as the high glucose conditions were

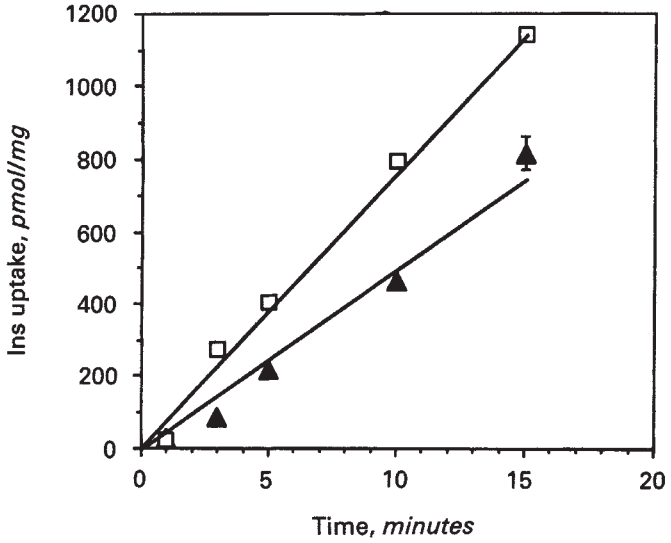

Fig. 1. *Effect of high glucose on inositol uptake in mesangial cells.* Cells were exposed to control (▲, 5 mM) or high glucose (□, 30 mM) conditions for 48 hours and inositol uptake was measured between 0 to 15 minutes; $P < 0.05$ for 5 versus 30 mM glucose at each time point between 3 and 15 minutes, $N = 9$.

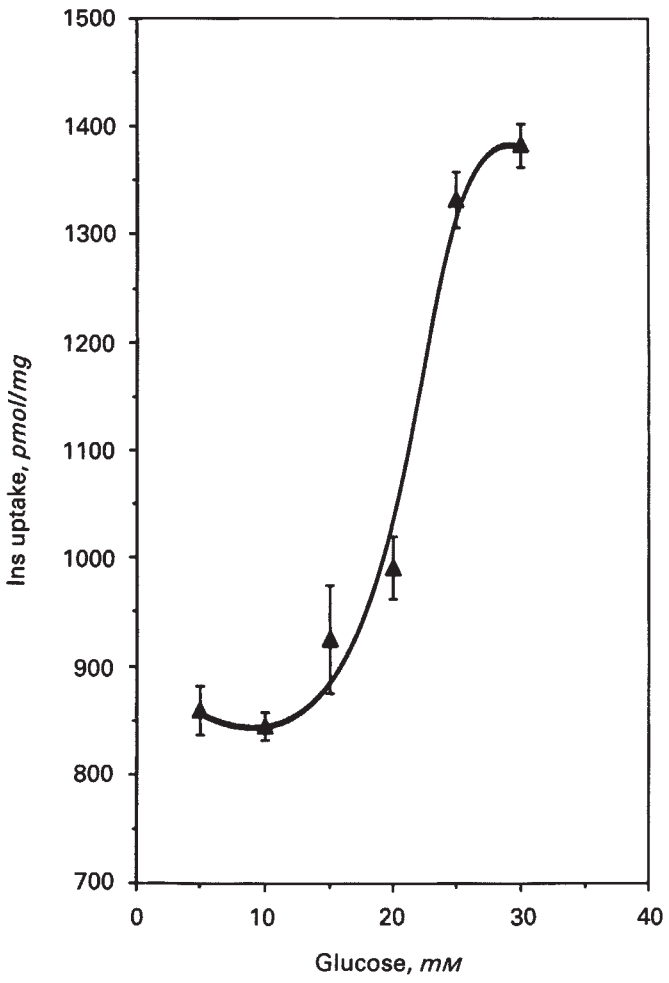

Fig. 2. *Effects of increasing concentrations of glucose on inositol uptake.* Mesangial cells were incubated for 48 hours in medium containing glucose in concentrations ranging from 5 to 30 mM and inositol uptake was measured over a 10 minute period. $N = 6$.

maintained (experiments terminated at 10 days) and was dependent on the concentration of glucose (Fig. 2). Incubation with similar concentrations of mannitol (osmolarity control) did not increase Ins uptake (Fig. 3). Thus, these results indicate that incubation of mesangial cells in high concentrations of glucose causes a concentration-dependent increase in the rate of Ins uptake and that this effect is specific for glucose and not due to changes in osmolarity.

To determine if the increase in Ins uptake induced by glucose was due to an increase in specific Na^+-dependent transport or due to an increase in nonspecific transport, Ins uptake was measured under Na^+-free conditions in cells exposed for 48 hours to normal or high glucose. Replacement of Na^+ with either choline or lithium resulted in an almost complete suppression of Ins uptake in cells exposed to both normal or high glucose conditions (Fig. 4). Thus, these results indicate that the increase in Ins uptake induced by high glucose is due to an increase in the activity of the specific Na^+-dependent Ins transporter and not due to nonspecific Ins transport.

To determine if the effects of high glucose on Ins uptake were the result of increased number of transporter units or of higher transporter affinity, V_{max} and K_m for Na^+-dependent Ins transport were determined using Lineweaver-Burke plots following exposure to normal or high glucose. Incubation with high glucose resulted in an increase in V_{max} (10.3 ± 0.2 vs. 16.4 ± 0.4 pmol/mg/min, for 5 vs. 30 mM glucose, respectively; $P < 0.005$) without change in K_m (10.2 ± 0.4 vs. 11.9 ± 0.6 μM, for 5 vs. 30 mM glucose, respectively; NS, $N = 3$). These results suggest that high glucose enhances Ins transport by increasing the number of Ins transporter units or through allosteric changes in transporter velocity but not by changing the affinity of the transporter.

To investigate if the induction of Ins transport by high glucose required synthesis of Ins transporter protein and transcription of mRNA, mesangial cells were exposed to normal or high glucose and coincubated with cycloheximide or actinomycin D for 24 hours. Both cycloheximide and actinomycin D prevented the increase in Ins transporter activity induced by high glucose (Fig. 5). Thus, these results indicate that glucose stimulation of Ins transport requires synthesis of new protein and suggest an effect at the gene transcription level.

Role of protein kinase C in the up regulation of Ins transport induced by glucose

We have previously demonstrated that elevated glucose induces an increase in membrane-associated protein kinase C as reflected by increased specific high affinity [^{3}H] phorbol 12,13-dibutyrate ([^{3}H] PDBu) binding in intact mesangial cells [5]. Since this technique measures the degree of protein kinase C translocation from the cytosol to its site of activation in the membrane and is considered to be a very precise index of protein kinase C activation [31, 32], our previous results likely reflect increased activation of the enzyme by glucose. This prompted us to further investigate a potential role of protein kinase C in the increase in Ins uptake induced by glucose. Since [^{3}H] PDBu binding is an index of protein kinase C translocation

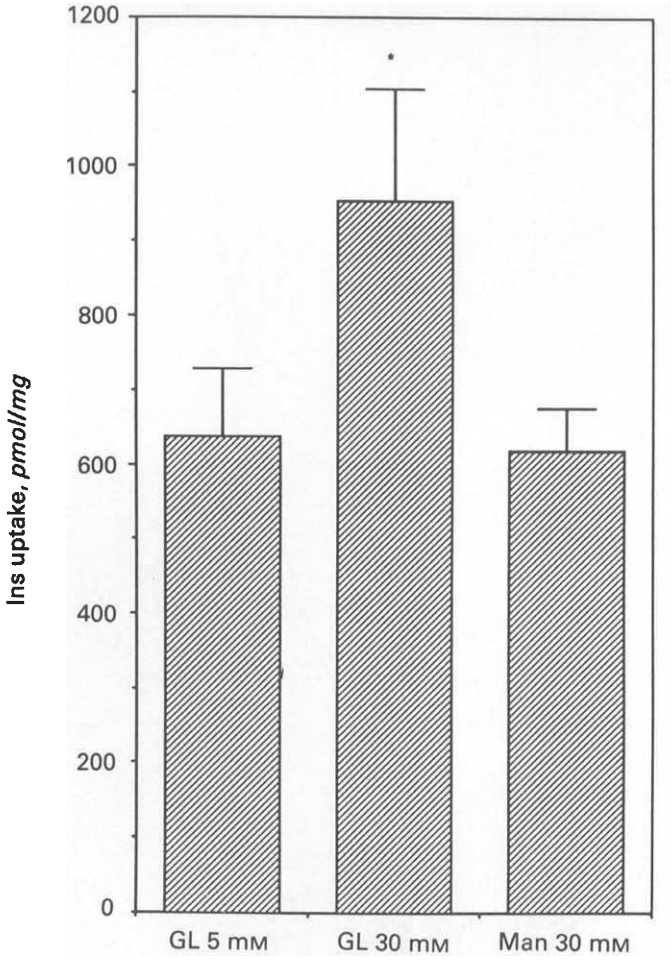

Fig. 3. *Effect of mannitol on inositol uptake in mesangial cells.* Cells were incubated for 48 hours in medium containing 5 or 30 mM glucose (GL), or 30 mM mannitol (Man). This was followed by determination of inositol uptake over a 10 minute period. * $P < 0.05$, $N = 6$.

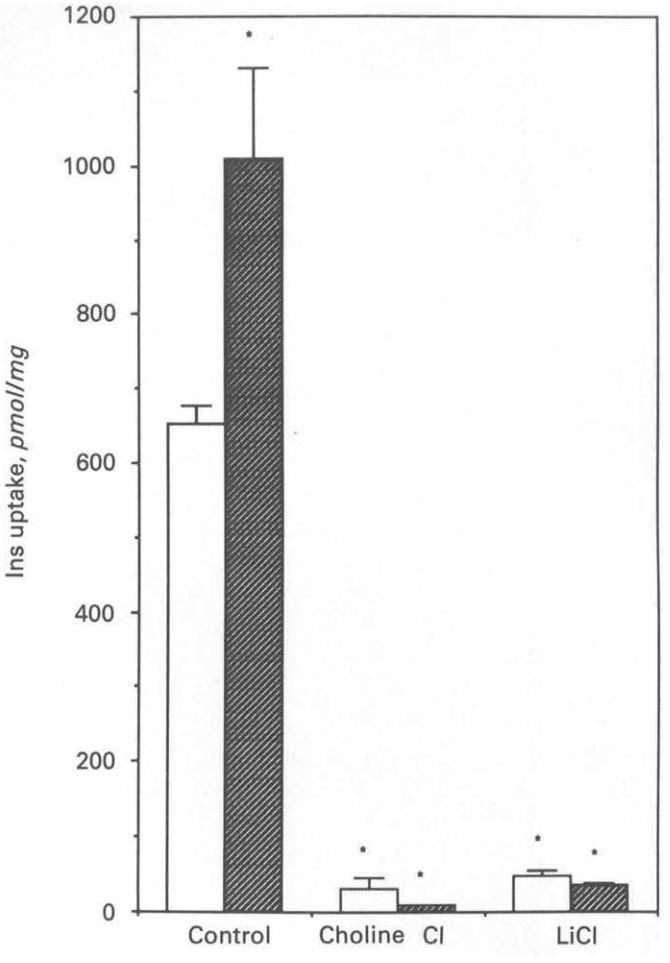

Fig. 4. *Inositol uptake in the presence (control) and absence of sodium in mesangial cells exposed to 5 (▥) or 30 mM (▨) glucose.* Uptake was measured over a 10 minute period. * $P < 0.05$ vs. glucose 5 mM in the presence of sodium (control), $N = 6$.

suggesting activation of the enzyme but does not measure activity, we decided to measure protein kinase C activity directly by phosphorylation of the highly specific synthetic peptide substrate $MBP_{4\text{-}14}$ as detailed above. As previously described by other investigators [30], we found that phosphorylation of $MBP_{4\text{-}14}$ after PMA treatment was dependent on peptide concentration and that it increased linearly with time. Preliminary experiments showed that, in our system, maximal phosphorylation occurred at a peptide concentration of 100 μM and that a time course of 15 minutes was adequate for measurement of protein kinase C activity (data not shown). Incubation of mesangial cells in high glucose caused an increase in protein kinase C activity as compared to controls (9.6 ± 0.6 vs. 11.2 ± 0.5 fmol/mg at 24 hr, 7.9 ± 0.4 vs. 11.8 ± 0.6 fmol/mg at 48 hr; 5 vs. 30 mM glucose, respectively; $P < 0.05$, $N = 4$; Fig. 6). This effect was specific for glucose since mannitol at similar concentrations did not activate the enzyme (data not shown).

To investigate the potential role of protein kinase C in mediating the effects of high glucose, Ins uptake was measured after direct activation of this enzyme with low concentrations of PMA (10 nM), and after inhibition with H7 or staurosporine in normal or high glucose. PMA stimulated Ins uptake in a fashion similar to high glucose, whereas H7 and staurosporine prevented the increase in Ins uptake induced by high glucose (Fig. 7). In addition, both H7 and staurosporine decreased basal levels of Ins uptake suggesting a role for protein kinase C in maintaining basal rates of transporter activity (in pmol/mg: control 636.2 ± 4.5; H7 356.9 ± 29.9; staurosporine 540.6 ± 16.2, $P < 0.05$ for either inhibitor versus control, $N = 6$).

Down regulation of protein kinase C was achieved by incubation with high concentrations of PMA. Mesangial cells were incubated with 1.6 μM PMA for periods of time ranging from five minutes to 16 hours and protein kinase C activity was determined. After initial stimulation following short term exposure to PMA, protein kinase C activity fell to basal levels within two to four hours and tended to remain below these thereafter (Fig. 8). These effects were similar in cells incubated in 5 or 30 mM glucose, and are in agreement with what has been previously reported in the literature [33]. Down regulation of protein kinase C with PMA resulted in a reduction in both basal and glucose-stimulated Ins uptake (Fig. 9). Thus, the results of these experiments demonstrate that high glucose causes an

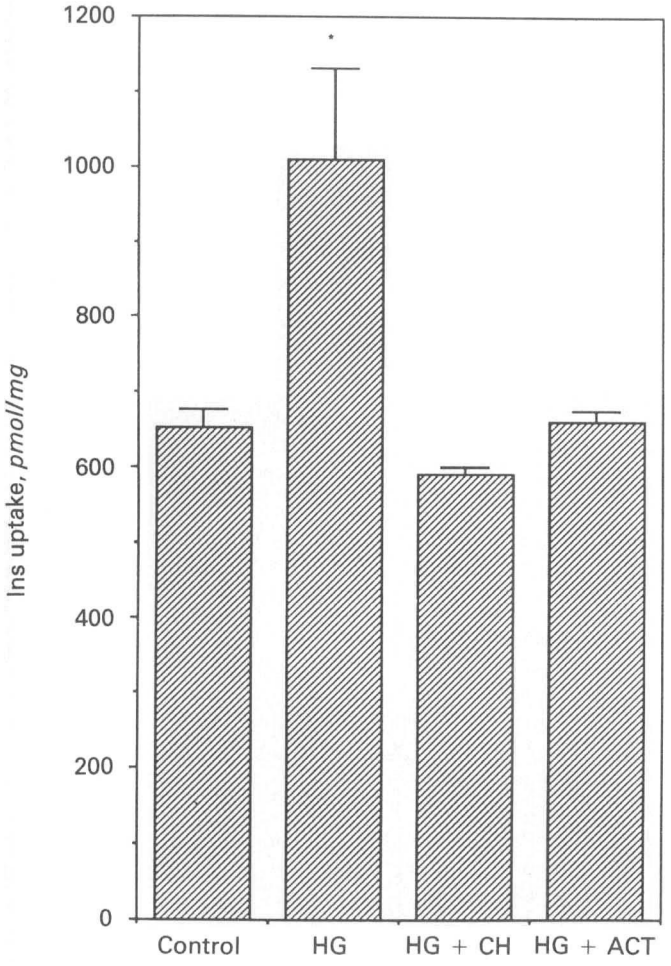

Fig. 5. *Effects of the protein synthesis inhibitor cycloheximide (CH, 20 μg/ml) and the mRNA synthesis inhibitor actinomycin D (ACT, 5 μg/ml) on the up regulation of inositol transport induced by high glucose.* Mesangial cells were incubated for 24 hours in medium contain 5 (control) or 30 mM (HG) glucose in the presence or absence of one of the above inhibitors. Inositol uptake was then measured over a 10-minute period as previously described. * $P < 0.05$, $N = 6$.

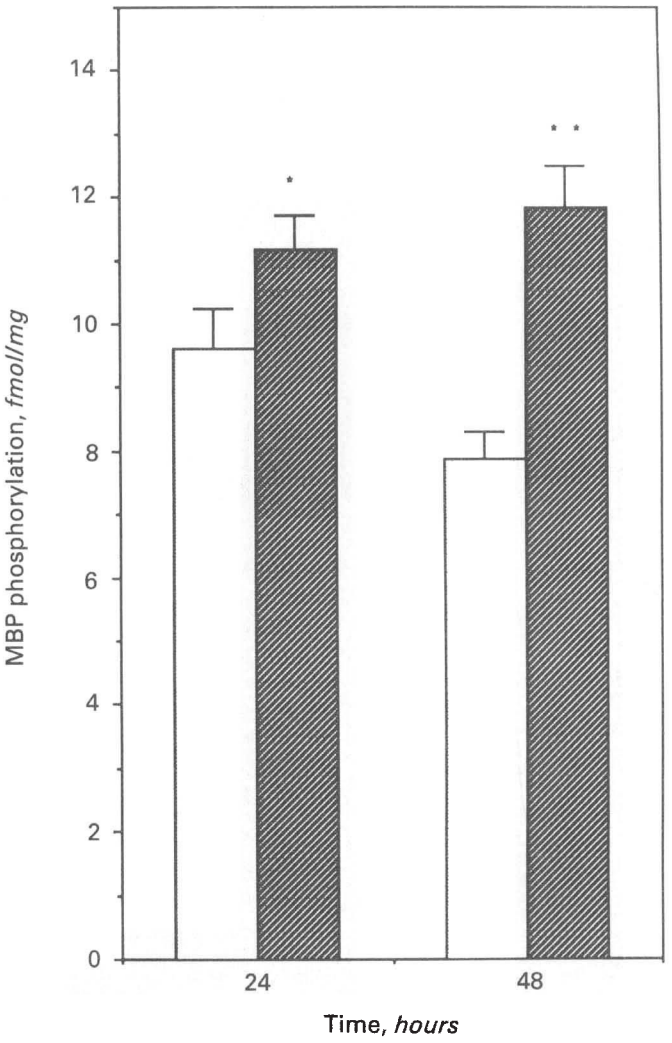

Fig. 6. *Protein kinase C activity measured as the rate of phosphorylation of MBP_{4-14} in digitonin-permeabilized mesangial cells.* Cells were incubated for 24 or 48 hours in medium containing 5 (▨) or 30 mM (▨) glucose and phosphorylation of MBP_{4-14} was then measured over a 15 minute period as described in the methods section. * $P < 0.05$, ** $P < 0.005$, $N = 4$.

increase in protein kinase C activity and that the up regulation of Ins transport by glucose requires activation of this enzyme.

Discussion

Ins deficiency has been proposed as one of the main pathogenetic mechanisms leading to tissue injury in diabetes mellitus [6–9]. A number of studies have characterized the kinetics of Ins transport in a variety of tissues and have investigated in detail the competitive nature of the inhibition of Ins transport by glucose [4, 16–23]. Little data is available, however, on the adaptive responses that come into play when this competitive interaction occurs. In these series of studies performed in rat glomerular mesangial cells in culture, we demonstrate that exposure to high extracellular concentrations of glucose results in stimulation of Ins transport via activation of protein kinase C. This effect is dose-dependent and specific for glucose, requires synthesis of new protein and is the result of an increase in the V_{max} of a specific Na^+-dependent Ins transporter. The stimulation of Ins transport by glucose is mediated by protein kinase C since the activity of this enzyme increases under high glucose conditions and the effect of glucose on Ins transport can be mimicked by treatment with PMA and inhibited by H7, staurosporine or by down regulation of protein kinase C.

We have previously shown that high glucose causes desensitization of receptor-mediated phosphoinositide hydrolysis in mesangial cells and that this is probably due to negative feedback by protein kinase C which becomes activated as a result of enhanced *de novo* diacylglycerol formation from glucose [5]. This desensitization is completely prevented by Ins supplementation which suggests that Ins may be playing a role in modulating protein kinase C activity. In fact, early studies have shown that supplemental Ins leads to increased shuttling of diacylglycerol into membrane phosphatidylinositol resulting in decreased intracellular levels of this lipid [34]. Furthermore, recent studies have suggested the existence in plasma membranes of a phosphatidylinositol synthetase which is responsible for the resynthesis of phosphatidylinositol during receptor

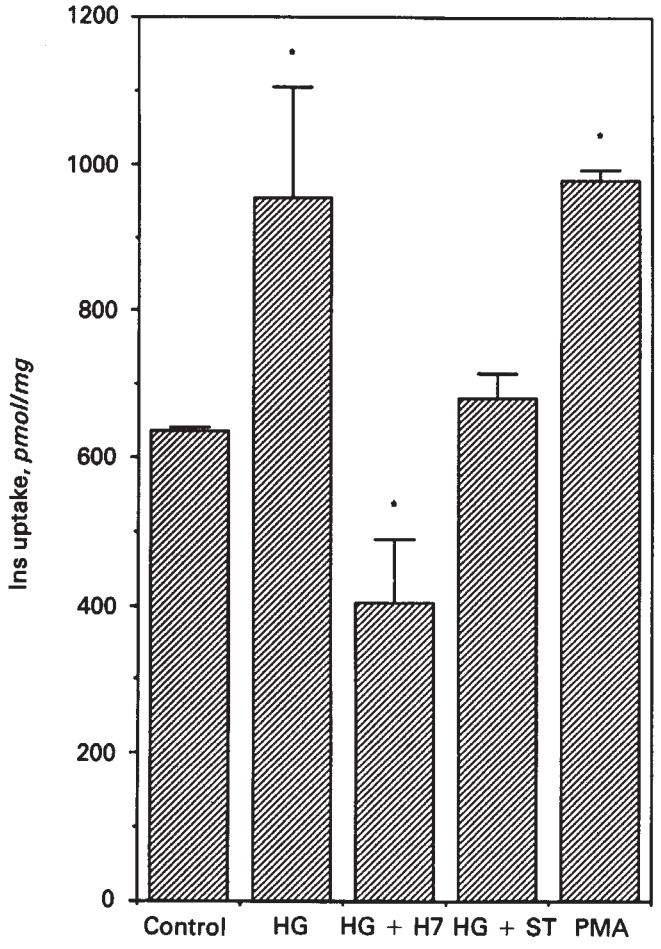

Fig. 7. *Effects of the protein kinase C inhibitors H7 (10 μM) and staurosporine (ST, 10 nM) on glucose-induced stimulation of inositol transport, and of PMA (10 nM) on basal inositol uptake.* Inositol uptake was measured over a 10-minute period following 48 hours of incubation in medium containing 5 mM glucose (control), 30 mM glucose (HG), 30 mM glucose plus the respective inhibitors, or 5 mM glucose plus PMA. Both H7 and staurosporine prevented the increase in uptake observed with high glucose, whereas PMA stimulated inositol uptake. * $P < 0.05$ vs. control, $N = 6$.

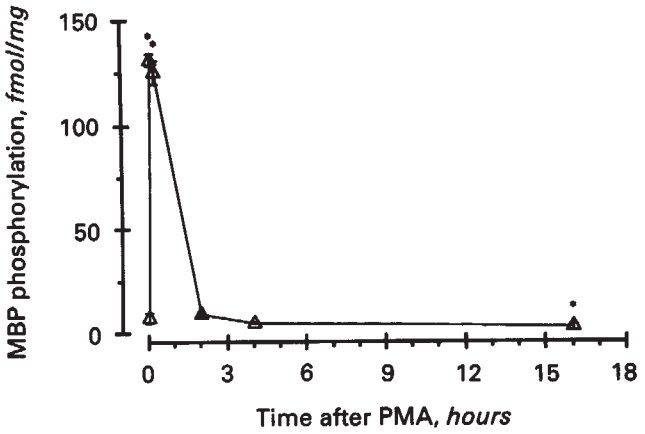

Fig. 8. *Down regulation of protein kinase C activity after prolonged treatment with PMA.* Mesangial cells were treated with 1.6 μM PMA for periods of time ranging from 5 minutes to 16 hours and protein kinase C activity was measured as described. After transient initial stimulation, protein kinase C activity fell and remained below basal levels. At 16 hours, the levels of protein kinase C activity in fmol/mg were: control 6.5 ± 1.0 and PMA 2.8 ± 1.3 ($P < 0.05$). Results shown are from cells incubated in 5 mM glucose. Similar results were obtained in cells incubated in 30 mM glucose. * $P < 0.05$ versus control. $N = 4$.

activation and has a K_m for Ins in the low micromolar range, similar to that of the transporter [35]. Conceivably, the Ins transporter could be closely linked to this phosphatidylinositol synthetase thus allowing Ins to rapidly shuttle diacylglycerol into phosphatidylinositol to replenish membrane phosphoinositide pools, and at the same time constitute a protective cellular mechanism by scavenging diacylglycerol and regulating the degree of protein kinase C activation.

Under conditions of chronic hyperglycemia, intracellular levels of Ins are reduced due to competitive inhibition of transport by high concentrations of glucose, and protein kinase C activity is increased as a result of *de novo* formation of diacylglycerol from glucose [4, 5, 36]. In this situation, increasing the rate of Ins uptake would tend to elevate intracellular Ins levels as an appropriate compensatory response for replenishing membrane phosphoinositide pools and scavenging diacylglycerol to limit protein kinase C activity. However, the increase in Ins transport may not be sufficient to overcome the sustained competitive inhibition of Ins transport by high concentrations of glucose. Thus in diabetes mellitus, the marked and sustained elevation in plasma glucose levels appears to overwhelm this compensatory increase in Ins transport such that total intracellular Ins remains depleted. Interestingly, although a number of studies have shown that the intracellular levels of Ins are depleted in diabetes mellitus and during in vitro hyperglycemia, when the levels of membrane phosphoinositides have been studied these have usually been found to be normal [5, 37]. It is therefore conceivable that, if as mentioned above, plasma membrane phosphatidylinositol synthetase is closely linked to the Ins transporter, the compensatory increase in uptake could preferentially replete those Ins pools that are of more critical importance in the moment to moment regulation of receptor function without actually normalizing total intracellular Ins levels. It is also interesting, however, that some studies have shown reversal of diabetic complications after dietary Ins supplementation [10], suggesting that if the extracellular concentrations of this sugar are sufficiently elevated, the deleterious effects of high glucose may be prevented, perhaps by mechanisms unrelated to the size of membrane phosphoinositide pools.

The fact that protein kinase C itself appears to regulate Ins transport under both basal and high glucose conditions raises the interesting possibility of this enzyme acting in a positive feedback loop to increase intracellular Ins levels which in turn would modulate enzyme activity. There is very little information available regarding the physiologic regulation of Ins transport in cells, and this study provides the first evidence that a major intracellular signaling system is involved in the up regulation of this transporter. It would be very interesting to examine the effects of hormonal agents and growth factors that act through the phosphoinositide cascade and protein kinase C on the physiologic regulation of the Ins transporter.

The mechanisms by which protein kinase C up regulates Ins transport are not known. Likely possibilities are that protein

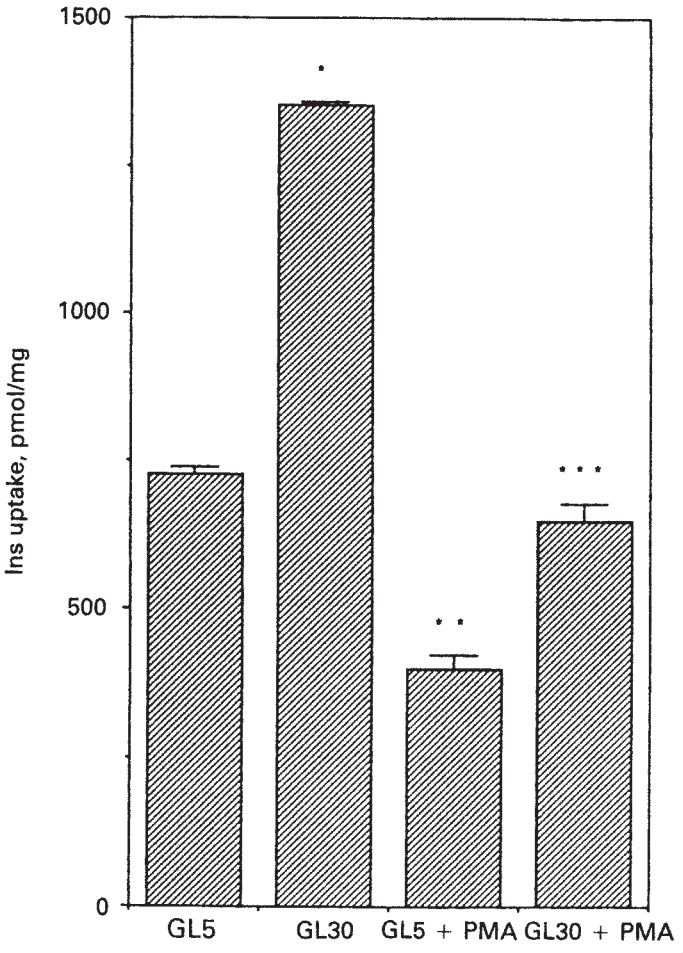

Fig. 9. *Effect of protein kinase C down regulation by prolonged incubation (16 hr) with PMA on glucose-induced stimulation of inositol transport.* Cells were incubated in medium containing 5 (GL5) or 30 (GL30) mM glucose in the presence or absence of 1.6 μM PMA and inositol uptake was measured over a 10-minute period. Incubation with PMA resulted in reduction in the basal levels of inositol uptake (GL5 + PMA vs. GL5, ** $P < 0.05$), and in marked blunting of glucose-induced stimulation of inositol uptake. PMA, however, did not completely abolish the effect of glucose (GL30 + PMA vs. GL5 + PMA, * $P < 0.005$ vs. GL5, *** $P < 0.05$). $N = 9$.

kinase C causes phosphorylation or stimulates the synthesis of Na^+/Ins cotransporters, or that it phosphorylates or stimulates synthesis of Na^+/K^+-ATPase thus providing more driving force for Na^+/Ins cotransport. The fact that the time course of maximal effect is long (24 hr) and that it requires protein synthesis makes it unlikely that a phosphorylation process is exclusively responsible for the increase in Ins uptake. Conversely, these observations coupled with the finding of increased V_{max} suggest that the effect of glucose is due to an increase in the number of transporter molecules and not to direct activation of the transporter by protein kinase C. Studies are under way to elucidate the precise mechanisms responsible for this effect.

In conclusion, exposure to high extracellular concentrations of glucose results in stimulation of Ins transport in cultured rat mesangial cells. This effect is specific for glucose and mediated by protein kinase C which becomes activated as a result of enhanced de novo diacylglycerol formation from glucose. This increase in Ins transport probably constitutes an adaptive response by which cells try to compensate for intracellular Ins depletion caused by glucose, and that may also serve as a mechanism by which more intracellular Ins is made available both to maintain membrane phosphoinositide pools, and to scavenge diacylglycerol and limit protein kinase C activity. Further studies should be conducted to better understand this interaction and other aspects of the physiologic regulation of Ins transport.

Acknowledgments

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References

1. Eagle H, Oyama VI, Levy M, Freeman AE: *myo*-Inositol is an essential growth factor for normal and malignant human cells in tissue culture. *J Biol Chem* 226:191–207, 1956
2. Holub BJ: Metabolism and function of *myo*-inositol and inositol phospholipids. *Ann Rev Nutr* 6:563–597, 1986
3. Rana RS, Hokin LE: Role of phosphoinositides in transmembrane signaling. *Physiol Rev* 70:115–164, 1990
4. Haneda M, Kikkawa R, Arimura T, Ebata K, Togawa M, Maeda S, Sawada T, Horide N, Shigeta Y: Glucose inhibits myo-inositol uptake and reduces *myo*-inositol content in cultured rat glomerular mesangial cells. *Metabolism* 39:40–45, 1990
5. Guzman NJ, Kurian P, Andaloro VJ, Tisher CC, Crews FT: Effects of glucose on receptor-mediated phosphoinositide hydrolysis and second messenger generation in rat glomerular mesangial cell. *J Am Soc Nephrol* 2:179–190, 1991
6. Greene DA, Mackway AM: Decreased *myo*-inositol content and Na^+-K^+-ATPase activity in superior cervical ganglion of STZ-diabetic rat and prevention by aldose reductase inhibition. *Diabetes* 35:1106–1108, 1986
7. Greene DA, Lattimer SA, Sima A: Sorbitol, phosphoinositides and sodium-potassium-ATPase in the pathogenesis of diabetic complications. *N Engl J Med* 316:599–606, 1987
8. Beyer-Mears A: The polyol pathway, sorbinil, and renal dysfunction. *Metabolism* 45:46–54, 1986
9. Beyer-Mears A, Ku L, Cohen MP: Glomerular polyol accumulation in diabetes and its prevention by oral sorbinil. *Diabetes* 33:604–607, 1984
10. Greene D, Lattimer S, Sima A: Are disturbances of sorbitol, phosphoinositide, and Na^+-K^+-ATPase regulation involved in pathogenesis of diabetic neuropathy? *Diabetes* 37:688–693, 1988
11. Weigensberg MJ, Garcia PF, Freinkel N: Uptake of *myo*-inositol by early-somite rat conceptus. Transport kinetics and effects of hyperglycemia. *Diabetes* 39:575–582, 1990
12. Baker L, Piddington R, Goldman A, Egler J, Moehring J: *Myo*-inositol and prostaglandins reverse the glucose inhibition of neural tube fusion in cultured mouse embryos. *Diabetologia* 33: 593–596, 1990
13. Hashimoto M, Akazawa S, Akazawa M, Akashi M, Yamamoto H, Maeda Y, Yamaguchi Y, Yamasaki H, Tahara D, Nakanishi T, Nagataki S: Effects of hyperglycaemia on sorbitol and *myo*-inositol contents of cultured embryos: Treatment with aldose reductase inhibitor and myo-inositol supplementation. *Diabetologia* 33:597–602, 1990
14. Kikkawa R, Kitamura E, Fujiwara Y, Arimura T, Haneda M, Shigeta Y: Impaired contractile responsiveness of diabetic

glomeruli to angiotensin II: A possible indication of mesangial dysfunction in diabetes mellitus. *Biochem Biophys Res Comm* 136:1185–1190, 1986
15. MAUER SM, STEFFES MW, ELLIS EN, SUTHERLAND DER, BROWN DM, GOETZ FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1155, 1984
16. KHATAMI M: Carrier-dependent and carrier-independent uptake of *myo*-inositol in cultured retinal pigment epithelial cells: Activation by heat and concentration. *Biochem Cell Biol* 66:942–950, 1988
17. KHATAMI H, ROCKEY JH: Regulation of uptake of inositol by glucose in cultured retinal pigment epithelial cells. *Biochem Cell Biol* 66:951–957, 1988
18. YOREK MA, DUNLAP JA, GINSBERG BH: *myo*-Inositol metabolism in 41A3 neuroblastoma cells: Effects of high glucose and sorbitol levels. *J Neurochem* 48:53–61, 1987
19. YOREK MA, DUNLAP JA, GINSBERG BH: Effect of sorbinil on *myo*-inositol metabolism in cultured neuroblastoma cells exposed to increased glucose levels. *J Neurochem* 51:331–338, 1988
20. SUSSMAN I, CARSON MP, SCHULTZ V, WU XP, MCCALL AL, RUDERMAN NB, TORNHEIM K: Chronic exposure to high glucose decreases *myo*-inositol in cultured cerebral microvascular pericytes but not in endothelium. *Diabetologia* 31:771–775, 1988
21. KOLLROS PE, GOLDSTEIN GW, BETZ AL: Myo-inositol transport into endothelial cells derived from nervous system microvessels. *Brain Res* 511:259–264, 1990
22. OLGEMOLLER B, SCHWAABE S, SCHLEICHER ED, GERBITZ KD: Competitive inhibition by glucose of *myo*-inositol incorporation into cultured porcine aortic endothelial cells. *Biochim Biophys Acta* 1052:47–52, 1990
23. YOREK MA, DUNLAP JA, LEENEY EM: Effect of galactose and glucose levels and sorbinil treatment on *myo*-inositol metabolism and Na^+-K^+ pump activity in cultured neuroblastoma cells. *Diabetes* 38:996–1004, 1989
24. HARPER PA, ROBINSON JM, HOOVER RL, WRIGHT TC, KARNOVSKY MJ: Improved methods for culturing rat glomerular cells. *Kidney Int* 26:875–880, 1984
25. LOVETT DH, RYAN JL, STERZEL RB: Stimulation of rat mesangial cell proliferation by macrophage interleukin-1. *J Immunol* 131: 2830–2836, 1983
26. MENE P, SIMONSON MS, DUNN MJ: Physiology of the mesangial cell. *Physiol Rev* 69:1347–1424, 1989
27. NAKANISHI T, TURNER RJ, BURG MB: Osmoregulatory changes in *myo*-inositol transport by renal cells. *Proc Natl Acad Sci USA* 86:6002–6006, 1989
28. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
29. HEASLEY LE, JOHNSON GL: Regulation of protein kinase C by nerve growth factor, epidermal growth factor, and phorbol esters in PC12 pheocromocytoma cells. *J Biol Chem* 264:8646–8652, 1989
30. YASUDA I, KISHIMOTO A, TANAKA S, TOMINAGA M, SAKURAI A, NISHIZUKA Y: A synthetic peptide substrate for selective assay of protein kinase C. *Biochem Biophys Res Commun* 166:1220–1227, 1990
31. TANAKA T, HAGIWARA M, HIDAKA H, NUNOKI K, OHTA H, ONODA K, ITO M, OHKUBO S, HIAI H, NISHIZUKA Y: Selective low level of protein kinase C isozyme in a tumor promoter-dependent mouse leukemia cell line. *Biochem Biophys Res Commun* 164:1397–1401, 1989
32. VACCARINO F, GUIDOTTI A, COSTA E: Ganglioside inhibition of glutamate-mediated protein kinase C translocation in primary cultures of cerebellar neurons. *Proc Natl Acad Sci USA* 84:8707–8711, 1987
33. BLACKSHEAR PJ: Approaches to the study of protein kinase C involvement in signal transduction. *Am J Med Sci* 296:231–240, 1988
34. FREINKEL N, EL YOUNSI C, DAWSON RMC: Inter-relations between the phospholipids of rat pancreatic islets during glucose stimulation and their response to medium inositol and tetracaine. *Eur J Biochem* 59:245–252, 1975
35. IMAI A, GERSHENGORN M: Independent phosphatidylinositol synthesis in pituitary plasma membrane and endoplasmic reticulum. *Nature* 325:726–728, 1987
36. LEE TS, SALTSMAN KA, OHASHI H, KING GL: Activation of protein kinase C by elevation of glucose concentration: Proposal for a mechanism in the development of diabetic vascular complications. *Proc Natl Acad Sci USA* 86:5141–5145, 1989
37. KANZAKI T, ISHIKAWA Y, MORISAKI N, SHIRAI K, SAITO Y, YOSHIDA S: Abnormal metabolism of polyunsaturated fatty acids and phospholipids in diabetic glomeruli. *Lipids* 22:704–710, 1987